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Increase in Peripheral Arterial Tone Predicts Myocardial Ischemia Induced by Mental Stress

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ABSTRACT

INCREASE IN PERIPHERAL ARTERIAL TONE PREDICTS MYOCARDIAL ISCHEMIA INDUCED BY MENTAL STRESS

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Mental stress ischemia (MSI) is associated with poor prognosis for coronary artery disease (CAD) and is amenable to treatment, yet no easily administered test exists to diagnose it. Given the known increase in systemic vascular tone in response to stress, we studied the ability of peripheral arterial tonometry (PAT), a noninvasive functional measure of arterial tone, to predict those vulnerable to MSI. Seventy-seven patients with chronic stable CAD were subjected to mental stress with concomitant assessment of myocardial perfusion and pulse wave amplitude. Nuclear perfusion imaging was used to document MSI, and PAT was used to measure pulse wave and microarterial tone. A ratio of PAT measurements during stress to those before stress was used to characterize vascular responses. Serum catecholamines and endothelin-1 (ET-1) were simultaneously measured. Subjects who experienced MSI had a lower average PAT ratio than those who did not (0.76 ± 0.04 vs. 0.91 ± 0.05, $P = 0.03$). A receiver operating characteristics curve for PAT ratio predicting MSI had an area under the curve of 0.613 (standard error, 0.065, one-sided $P = 0.04$). Maxima of sensitivity and specificity were observed at a threshold of 0.78 to define an abnormal PAT ratio. Cross-tabulation of groups above and below this threshold with groups of subjects with and without MSI showed a significant predictive relationship between PAT ratio and MSI ($P = 0.03$). Subjects at or below this threshold (≤ 0.78) displayed a significant increase in norepinephrine levels during mental stress (235 pg/ml at baseline, 259 pg/ml during mental stress, $P = 0.007$). Subjects above this threshold (>0.78) displayed a significant decline in their ET-1 levels 24 hours after mental stress (1.15 pg/ml after mental stress, 0.93 pg/ml 24 hours later, $P = 0.01$), while those at or below threshold had a continued increase. PAT ratio is a complex functional measure of peripheral arterial tone that significantly predicts the occurrence of MSI. It may have clinical value as an easily administered screening test for MSI.

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INTRODUCTION

Acute mental stress has long been known to have detrimental effects on the cardiovascular system. Acute episodes of stress due to a dramatic event, such as the death of a loved one or a natural disaster, are anecdotally associated with fatal cardiovascular events in popular lore. In fact, the experience of acute psychological, psychosocial, or emotional stress can precipitate acute coronary syndromes (ACS), such as myocardial infarction (MI), and it can also cause fatal arrhythmias. Studies of the association between anger and coronary artery disease (CAD) have established a demonstrable link between the two phenomena (1, 2). However, the physiological mechanisms underlying these associations are poorly understood.

As the effects of acute stress on the cardiovascular system have been studied, it has become apparent that infarctions and arrhythmias culminating in sudden cardiac death are only the most dramatic manifestations of these effects. Those vulnerable to these outcomes may manifest mental stress-induced pathology in less dramatic ways. Acute mental stress causes silent myocardial ischemia in at least 30%, and in some studies as many as 60%, of patients with CAD who are subjected to an acute laboratory stressor (3). This mental stress ischemia (MSI) is generally asymptomatic and unapparent to the afflicted individual, and thus there are often no warning symptoms. Nevertheless, it can cause observable pathology, including abnormalities of the motion of the ventricular wall and a fall in left ventricular ejection fraction (LVEF), which compromise the efficiency of the heart as a pump. It can also lead to deficits of myocardial perfusion that may be seen with nuclear imaging techniques and in some

cases can cause electrophysiological changes in the heart that can be seen on electrocardiography (ECG) and can contribute to arrhythmia.

The occurrence of MSI in vulnerable individuals with CAD is also associated with poor prognosis for cardiovascular outcomes relative to those who do not experience it (4, 5). MSI may be a marker for more advanced CAD, merely indicating those individuals in whom the disease will progress more rapidly or be more severe. But MSI may also directly contribute to CAD progression through damage caused by repeated episodes of subclinical ischemia. MSI is silent and subtle, but the ability to evoke it consistently in the laboratory suggests its frequent occurrence in everyday life, with attendant consequences.

Despite the clinical significance of MSI, there is currently no easily administered and noninvasive way either to observe it or to identify those who are vulnerable to it. Trans-thoracic echocardiography (TTE) can be used to observe ventricular wall motion in patients undergoing a mental stress task, but not all patients with ischemia will manifest it as a wall motion abnormality (6). Furthermore, the presence of a wall motion abnormality may be related to increased afterloading of the ventricle due to the rises in blood pressure and systemic vascular resistance (SVR) that mental stress elicits (7) and therefore may not be specific to myocardial ischemia. The "gold standards" for observing myocardial ischemia, coronary angiography and nuclear perfusion imaging, require either invasive techniques or noninvasive administration of radioactive isotopes to assess perfusion. Consequently, the utility and application of mental stress testing as a tool for assessing cardiovascular risk remain controversial, although its prognostic value has been shown (8). This is in spite of the fact that emerging treatment strategies to aid

those who are vulnerable to MSI are effective in reducing the severity, progression and cost of cardiovascular disease (9-11).

Epidemiology

Perhaps our earliest and most intuitive understanding of the role of acute stress in CVD has come from the association of MI with the experience of sudden emotional stress, particularly anger. This anecdotal association has been shown to have a scientific basis. For example, a study of reported anger in victims of a recent nonfatal MI demonstrated a 2.5-fold increased risk of MI for up to 2 hours after the experience of moderate to extreme anger (1), while a prospective study of anger control found that those who reported high levels of anger had a 3.15 relative risk of MI compared to those who reported low levels during a seven year follow-up period (2).

Earthquakes have served as a useful example of an acute mental stressor experienced by many people in a similar manner. The potential of such a stressor to provoke MI had been reported as early as 1983, in a study of fatal heart attacks after the Athens earthquake of 1981 which found that deaths attributable to coronary disease increased significantly to 7.7 fatalities per day in the three days following the earthquake from a background CAD mortality of 2.6 fatalities per day (12). Subsequent studies of the 1994 Northridge, California, earthquake confirmed the association between these two phenomena (13, 14). Additionally, victims of the 1999 Taiwan earthquake were found to have perturbations of heart rate variability (HRV) in the period immediately following the quake, which may contribute to arrhythmogenesis and sudden cardiac death (15).

Mental stress may directly cause cardiac arrhythmias as well. Patients experiencing psychological stress can experience life-threatening ventricular arrhythmias in the absence of underlying structural heart disease, an effect which may be related to increased sympathetic activation (16). Two studies examined the triggering of shocks to correct ventricular tachyarrhythmias in patients with implanted cardioverter-defibrillators (ICDs) after the terrorist attacks of September 11, 2001. These studies found that, in the weeks immediately following the attacks, ventricular arrhythmias increased significantly in patients both proximal to and far removed from the locations where the attacks occurred (17, 18). These findings indicate that the deleterious effects of acute mental stress are not limited to stressors that pose an immediate, tangible risk, as in the example of an earthquake. Mental stress unrelated to a proximate physical threat, when acutely experienced, is sufficient to trigger cardiac arrhythmia.

The effects of an acute episode of emotional stress are not limited to the hours or days immediately following the exposure. One long-term prospective study of individuals who had lost a child found that their relative risk of a fatal MI from 7 to 17 years after the event was 1.58 compared with matched controls, suggesting that, in addition to triggering acute cardiac events, a severe stressor may contribute to long-term mortality as well, possibly through chronic sequelae arising from an acute, emotionally traumatic experience (19).

A syndrome of acute heart failure in response to a severe, acute mental stressor has been described in several case reports and case series (20, 21). It is marked by severe ventricular dysfunction and wall motion abnormalities. In contrast to precipitation of MI or arrhythmia, in which the acute stressor exploits the already diseased heart and

coronary arteries, this syndrome may occur in otherwise healthy individuals who lack evidence of significant coronary or electrophysiological disease. It therefore appears to occur directly as a result of the stressor. This syndrome of myocardial stunning after acute mental stress is not yet well understood. There is some evidence that it may be caused by extreme activation of the sympathetic nervous system and marked by supra-physiological levels of catecholamine release. At such high levels, the catecholamines become cardiotoxic and appear to induce the syndrome (21).

Cardiac-related deaths that are directly attributable to acute mental stress are nevertheless relatively rare. The occurrence of subclinical MSI is likely far more common, as indicated by studies that have documented ischemia either in response to a laboratory stress task or in an individual's normal environment using ambulatory ECG monitoring. The Psychophysiological Investigations of Myocardial Ischemia (PIMI) Study studied 196 men and women with stable CAD and found evidence for MSI in 58% of them using a combination of two stressors. Only a small fraction (10%) of those with MSI also reported angina during the stress tasks, while 8% displayed ST-segment depression on ECG (7).

Another study of 132 patients with CAD found a prevalence of MSI of 34%, and also found that those with MSI in the laboratory were more likely to exhibit ischemia during daily life on ambulatory monitoring (relative risk 2.98 versus those without MSI) (22). In specifically addressing the association of ambulatory ischemia with mental stress, another study found an adjusted relative risk of 2.2 for transient ischemia in the period immediately following the experience of negative emotions (23). Other studies

using smaller numbers have found MSI to occur in 39% (24), 43% (25) and 60% (26) of patients.

The occurrence of MSI has prognostic implications. In a longitudinal study, 30 patients with stable angina pectoris and ischemia on stress perfusion imaging underwent continuous left ventricle functional monitoring during stress induced by serial subtraction. They were then followed for two years for occurrences of MI or unstable angina. Of the 15 patients with LV dysfunction during stress, 10 went on to experience one of these events during the follow-up period, while only 4 of the 15 patients without LV dysfunction experienced an event (4). This finding has been replicated by three other studies (5, 27, 28), with the most recent demonstrating a statistically significant 3.0 rate ratio for death over a 5-year follow-up among CAD patients with ischemia during laboratory mental stress (5).

Laboratory Studies of Mental Stress and Myocardial Ischemia

In 1984, Deanfield and colleagues reported that a mental stress task performed in the laboratory could provoke asymptomatic myocardial ischemia (29). By subjecting patients with stable angina to a mathematical arithmetic stress task, the investigators were able to elicit abnormalities of myocardial perfusion using Rb-82 positron tomography. In 50% of those demonstrating ischemia, there were no accompanying symptoms or obvious ECG changes. This phenomenon has been extensively observed in patients with CAD since then and, as described, has been found to occur in anywhere from 30-60% of patients with CAD (3).

A number of methods have been used both to provoke MSI and to observe it. Among the provocations, or tasks, that have been more commonly used are mental arithmetic, the Stroop color-word task, public speaking, anger recall, and anger recall with desperation or helplessness. These vary in terms of their cognitive demand and emotional component; however, all have been found to elicit MSI. The criteria used to define MSI have already received some attention and include the occurrence of a new ventricular wall motion abnormality, a decline in LVEF, and the presence of qualitative or quantitative perfusion defects identified with nuclear perfusion imaging of the myocardium.

Mental arithmetic generally involves asking a subject to subtract a number serially from another number (e.g., 7 from 999, so that the series of correct answers is 992, 985, 968, etc.) while the person administering the task berates and rushes the subject to produce a certain error rate. The element of pressure and public performance combines with a cognitive challenge to produce stress.

The Stroop color-word task puts the name of a color on a computer screen but prints that name in a discordant color (e.g., the word "red" in yellow text). The subject is then asked to respond with the color of the text, not the color actually named (in the example, the correct response would be "yellow"). The responses are timed in order to introduce an element of time pressure to the task, and advanced versions will titrate the difficulty and speed of the task based on the subject's performance.

Public speaking asks the subject to give a brief speech to one or more evaluators on a topic that has some emotional valence, such as a difficult interpersonal interaction.

The stress produced involves both the nature of the topic and the pressure of speaking in front of the evaluators.

Anger recall functions similarly except that the subject chooses a recent experience that provoked irritation, aggravation or anger. The subject recounts this experience to an interviewer who also asks leading questions and encourages the subject to explore their feelings and actions during the experience. When desperation is included in the task, the recent experience chosen must include an element of helplessness on the part of the subject, a feeling that they could not affect or ameliorate the situation.

While all of these tasks can produce MSI, anger recall may be the most potent in eliciting MSI (24) and has been found to be most successful in reproducing MSI in a given subject (30).

The methods for observing MSI and the corresponding criteria to define them have advantages and disadvantages. Mental stress has the ability to cause local abnormalities in cardiac wall motion during the ventricular beat (25, 31). LVEF and wall motion can be assessed noninvasively with TTE, or by nuclear imaging with use of intravenous radionuclides. However, the decline in LVEF that occurs during mental stress is in part attributable to an increase in SVR. This is also likely true for wall motion abnormalities, as previously mentioned, and so these may result from increased afterloading, from segmental myocardial ischemia, or from a combination of the two. Consequently, these traditional measures of MSI may not be specific to myocardial ischemia *per se*.

One study that used simultaneous perfusion imaging of the myocardium and TTE to observe MSI found that concordance between the two methods was only 46% (6).

This finding suggests that ventricular dysfunction during mental stress may not be attributable to decreased perfusion in a significant number of cases and may in fact result from afterloading or other unknown causes. Perfusion imaging may therefore represent a "gold standard" for observation of MSI. It provides the ability to identify ischemia during mental stress definitively. Its disadvantages include the fact that it requires the intravenous administration of radioactive isotopes, and that it is expensive.

The deficits of function and perfusion that constitute MSI are accompanied by deficits of vasomotor function in the coronary arteries during mental stress. In 1991, Yeung and colleagues reported that, in diseased coronary arteries, a mental arithmetic stress task caused vasoconstriction that decreased coronary blood flow. The degree of constriction correlated with paradoxical constriction of diseased arteries in response to an infusion of acetylcholine (32). Such abnormal responses to normally vasodilatory stimuli have come to be understood as endothelial dysfunction, so-called because they represent a deficiency of endothelium-dependent relaxation of vascular smooth muscle. This phenomenon has been observed in peripheral arteries as well (33, 34), using brachial artery reperfusion-dilation (commonly called flow-mediated dilation or FMD) as a surrogate measurement that correlates with coronary endothelial dysfunction (35).

Mental stress also affects the electrophysiology of the heart and may contribute to cardiac arrhythmias. It can both cause and destabilize ventricular arrhythmias and has been shown to worsen ventricular tachycardia in the laboratory (36, 37). Myocardial ischemia changes the conductance of sodium and potassium across the cell membrane, and thus alters the electrical potential of the myocardial cell. This can lead to instability

of the myocardium and cause the cells to fire in a disordered manner instead of in a unified pattern, predisposing to pathological arrhythmias.

Mental stress may induce silent myocardial ischemia, but its effects on the coronary arteries may also be even more subtle. In some patients with CAD, the response to mental stress may be marked not by ischemia but by a relative diminution of coronary function. While these subjects do not become frankly ischemic, they do not exhibit the same increase in myocardial blood flow that accompanies mental stress in healthy subjects, characterized as coronary flow reserve. One study documented this phenomenon in 10 subjects with CAD using quantitation of absolute myocardial blood flow. It found that coronary flow reserve was diminished in these subjects during mental stress relative to a control group (38). This diminution was most pronounced in those myocardial segments in which no critical stenosis of the epicardial coronary arteries was observed on coronary angiography. In other words, a mechanism other than critical, flow-limiting stenotic disease was causing this failure of the coronary arteries to normally augment myocardial blood flow. It has been postulated that this effect may result from dysfunction of the coronary microvasculature, dysfunction that may contribute to MSI (38). These findings in combination with earlier findings of vasoconstriction in epicardial arteries (32) suggest two distinct mechanisms for MSI in the coronary circulation.

Physiology and Pathophysiology

Research is moving beyond the descriptive effects of mental stress on the cardiovascular system and into a nascent understanding of the pathophysiology of mental

stress. This work encompasses all areas of the experience of mental stress, from its initial processing and manifestations in the central nervous system (CNS) to its ultimate effects on the heart and blood vessels.

The various systems in the brain responsible for interpreting and manifesting the experience of stress are being described through the use functional neuroimaging modalities in humans. Differences in mood and emotional coping mechanisms among human subjects make it implausible to detect highly consistent patterns of cortical activation across different neuroimaging studies of mental stress. Furthermore, different induction methods of mental stress through cognitive and emotional tasks elicit activity in varying areas of the brain (39). Nevertheless, the totality of neuroimaging studies in healthy normal subjects imply that the stress response is mediated by limbic structures—such as the amygdala, hippocampus, hypothalamus, and cingulate cortex—in addition to prefrontal regions, the insular cortex, and other brainstem nuclei (39-41). These results point to a complex, integrated response involving centers dealing with emotion, reason, affect, and executive function. Furthermore, some of these areas are implicated in autonomic control of the cardiovascular system (42), and so these findings are significant because our understanding of the subsequent effects of mental stress—after it has been processed and interpreted as stress—focuses on the autonomic nervous system and its physiological effects.

These findings integrate with—and in a number of cases form a part of—a putative central autonomic network that mediates the response to stress in the CNS, originally posited by Benarroch (43). This network forms the basis for a model of neurovisceral integration in mental stress elaborated by Thayer and Lane (44), which

proposes mechanisms by which the CNS, autonomic nervous system (ANS), and peripheral systems (e.g., inflammatory responses, regulators of vascular tone) integrate and modulate the response to stress. These systems then regulate the output of stress effector systems like the hypothalamic-pituitary-adrenal (HPA) axis and the ANS. In the case of mental stress, the net effect of activation of this system may be an imbalance in autonomic tone marked by increased sympathetic output and decreased parasympathetic output (45). This imbalance may contribute to cardiovascular pathology caused by mental stress.

The output of the HPA axis and the ANS can be measured either by surrogate measures of efferent tone, or by direct measurement of their component molecules. Examples of methods that employ these two strategies include muscle sympathetic nerve activity (MSNA), an invasive method of measuring sympathetic tone; HRV, the high-frequency component of which can be used as a non-invasive measure of vagal, or parasympathetic, tone; direct measurement of plasma and saliva cortisol, the latter providing a convenient and noninvasive method for measuring HPA output; and direct measurement of serum catecholamines, the primary mediators of sympathetic output.

Acute mental stress is known to cause elevations in several measures of cardiovascular function, including heart rate, blood pressure, rate-pressure product, and cardiac index (46). What is unusual about acute mental stress, in contrast to exercise stress, is that it is accompanied by an increase in SVR not seen during exercise that contributes to a concomitant drop in LVEF in healthy individuals without evidence of CVD (46). This phenomenon also occurs in patients with CAD (7, 25).

These increases in hemodynamic parameters reflect an increase in vascular tone during mental stress and are accompanied by an increase in sympathetic tone as measured by both catecholamine levels and MSNA. The PIMI Study measured sympathetic responses to various mental stressors by measuring serum catecholamines in both the study group of subjects with CAD and in a reference group of 29 healthy subjects (7, 46). Both groups experienced increases in serum catecholamines during mental stress, and epinephrine in particular was correlated with increases in blood pressure, heart rate, cardiac output, and SVR in the CAD group (7). Serum cortisol was measured in the reference group during the acute stress tasks, and no significant differences were found in cortisol levels between baseline and any of the acute stress conditions. However, other studies have shown salivary cortisol to be increased in the setting of chronic job stress and ongoing daily stressful events (47, 48). In fact, a great deal of evidence points to an important role for cortisol in mediating the effects of chronic stress. Its role in contributing to pathology caused by acute stress is poorly understood, and there is little evidence that it does so. Consequently, it is not being studied in this research project.

Using MSNA as well as measurements of heart rate and blood pressure, one group of researchers assessed responses to mental stress mediated by sympathetic tone by blocking sympathetic output centrally with the drug monoxidine. They found that increases in heart rate, blood pressure, and MSNA during a mental arithmetic stress task were all attenuated by a central sympathetic blockade (49). These findings in combination with those of the PIMI study and other studies point to an essential role for sympathetic output in mediating acute responses to mental stress.

The role that mental stress plays in causing arrhythmias appears to have both sympathetic and parasympathetic components. Mental stress likely destabilizes the normal electrophysiology of the myocardium, and this destabilization apparently occurs through effects on two electrophysiological parameters. First, heart rate variability (HRV) is decreased by mental stress, likely through a pathway mediated by the CNS that affects vagal tone and therefore beat-to-beat period variation (50). Second, abnormal myocardial repolarization appears to increase during mental stress, as measured by indices of T-wave parameters on ECG that correspond to heterogeneity of repolarization (51). Increases in T-wave alternans, a particular measure of heterogeneous repolarization, correlate with increases in epinephrine seen during mental stress and may increase the likelihood of developing an arrhythmia (51). Interestingly, the study that demonstrated this relationship found that changes in myocardial repolarization did not correlate with changes in parasympathetic tone as measured by high-frequency HRV. This suggests that mental stress may contribute to arrhythmogenesis through an effect of sympathetic mediators on the myocardium itself, as well as through an effect of parasympathetic tone on rate control and pacing. The contribution of decreased parasympathetic tone to pathology caused by mental stress in CAD remains unclear, although there is some early evidence that parasympathetic tone may play a protective, anti-inflammatory role in CVD (52).

The consequences of mental stress-induced activation of these neurohormonal pathways are therefore in at least four parts. First, chronic mental stress leads to a reduction in parasympathetic tone, which could then promote a pro-inflammatory state in the vessels that damages the endothelium and contributes to atherogenesis (52). Second,

mental stress increases both blood pressure and SVR, leading to increased afterloading of the ventricle, which may have both acute and chronic effects on its function as a pump. Third, changes in autonomic tone affect vascular tone, leading to vasoconstriction or even vasospasm in diseased large arteries and in the microcirculation. Ischemia and myocardial damage then ensue as a direct result of vasoconstriction, plaque disruption, or both. Finally, changes in the balance of autonomic tone affect the electrophysiology of the heart. This predisposes the heart to fatal arrhythmias which can cause sudden death (37, 51). These mechanisms may occur independently or simultaneously. In the latter case, it is likely that their effects are synergistic. For example, inflammatory changes may make vessels more susceptible to sympathetic vasoconstriction; the resulting local ischemia and myocardial irritation may in turn lead to arrhythmogenesis. Increased sympathetic tone during mental stress may independently perturb normal vascular function, or it may take place in the setting of disordered endothelial responses and may exploit a damaged endothelium to cause MSI. In examining these relationships, the connection between endothelial dysfunction and mental stress will be discussed, and then the possible interaction or interdependence of sympathetic and endothelial mechanisms in causing MSI will be considered.

Endothelial Dysfunction and Endothelin-1

The endothelium comprises a single layer of cells that lines the lumen of all blood vessels, serving as a bi-directional, biocompatible barrier that facilitates the passage of blood gases and a range of molecules to and from tissues. The endothelium maintains vascular homeostasis, responding to circulating and hemodynamic factors by releasing

bioactive substances to affect vascular tone, with the vasodilator nitric oxide (NO) as the primary active agent (53). Endothelial dysfunction may be viewed as the failure of the endothelium to respond appropriately to these circulating and hemodynamic factors. This dysfunction provides the setting for paradoxical vasoconstriction, platelet activation and leukocyte adherence, thrombosis and vascular inflammation, and eventual atherosclerosis (54). Endothelial dysfunction is a product not only of decreased NO levels and decreased activity of the enzymes (e.g., endothelial nitric oxide synthase or eNOS) that synthesize it, but also of the resulting unopposed vasoconstriction. The endothelium-dependent mechanism that contributes to this effect is mediated by endothelin-1 (ET-1) (55).

ET-1 is one of a family of vasoactive polypeptides including as its primary isoforms ET-1, ET-2, and ET-3 (56). ET-1 is a 21-amino acid peptide produced primarily by the endothelium (57) but also by other cell types, including vascular smooth muscle cells (VSMCs) (58) and leukocytes (59). It is one of the most powerful endogenous vasoconstrictors known (57). It produces vasoconstriction by causing influx of calcium ions into VSMCs, an effect that is mediated by stimulation of G protein-coupled endothelin receptors (60). It is also a mitogen involved in endothelial, VSMC, and glial cell proliferation, acting through two distinct mitogen-activated protein kinase cascades to produce these effects (60). Both of these actions are predominantly mediated by the endothelin-A (ET_A) receptor subtype. ET-1 can also paradoxically produce vasodilatation by stimulating endothelin-B (ET_B) receptors on endothelial cells, which stimulate eNOS to produce NO. This appears to provide an important negative feedback pathway for limiting the vasoconstriction caused by ET-1 activity at ET_A receptor sites. Additionally, stimulation of the ET_B receptor by ET-1 is involved in mitogenic

stimulation of the endothelium (61) but may have pro-apoptotic effects in other cell types, such as melanocytes (62).

The vasoconstrictor ET-1 is functionally related to NO, working in concert with and in opposition to NO in the maintenance of vascular tone. It has been shown that endothelial dysfunction results not just from decreased levels of NO but also from increased activity of ET-1 (55). This reflects an imbalance of vasoconstricting and vasodilating substances being produced by, and acting on, endothelial cells, an imbalance which itself indicates endothelial dysfunction (63).

Measurement of Endothelial Dysfunction

As endothelial dysfunction can reflect imbalances or changes in levels of various vasoactive molecules, measurement of levels of these molecules has become an increasingly accepted way to document dysfunction, as their levels have been found to be higher in those with endothelial dysfunction compared to those with normal endothelial function. Examples of these molecular markers include certain cytokines, adhesion molecules, and metalloproteinases. ET-1 itself is also a marker for dysfunction; elevated levels are associated with endothelial dysfunction and are found in those with documented atherosclerosis (64, 65).

Endothelial dysfunction has traditionally been measured in a few different ways (63). The "gold standard" measurement of cardiovascular endothelial dysfunction is via catheter infusion of acetylcholine into the coronary arteries during coronary angiography. In healthy arteries, this produces endothelium-dependent vasodilatation, while in the arteries of those with CAD, it produces paradoxical vasoconstriction, even in arteries

without apparent stenotic disease (66, 67). This technique provided some of the earliest documentation of endothelial dysfunction occurring during mental stress (32).

FMD of the brachial artery is a common peripheral, surrogate technique (68). The brachial artery is occluded transiently by a blood pressure cuff and then reperfused. The vasodilatation that results occurs through endothelium-dependent mechanisms, particularly the effects of mechanical distention and shear stress on the reperfused vessels, which stimulate NO production (69, 70). A stereotactic ultrasound probe is used to record the diameter of the brachial artery before, during, and after the reperfusion, with changes in these values during an intervention or test referenced to the baseline values for a subject (63). This method requires a very high degree of operator skill and expertise in order to produce reproducible measurements. These considerations likely preclude its viability as a clinical diagnostic tool (63).

Finally, assessment of endothelial dysfunction in the smaller arteries of the forearm and digits has been performed using both laser Doppler flowmetry of the microcirculation and strain gauge venous plethysmography, which measures changes in venous distention caused by changes in forearm blood flow. These methods are even more vulnerable to operator variability than FMD and their use has been limited to exploratory research studies (63).

Endothelial Dysfunction and Endothelin-1 during Mental Stress

Mental stress can cause endothelial dysfunction in healthy individuals (71), and selective blockade of the ET_A receptor has been shown to reverse the endothelial dysfunction observed during mental stress (72), suggesting a unique role of ET-1 in

mediating vascular responses and endothelial dysfunction during mental stress. ET-1 levels have been shown to rise abruptly after a brief period of mental stress in the circulations of both young healthy volunteers (73) and patients with vascular disease (74). In addition, the increase in circulating ET-1 levels seen during mental stress is greater in offspring of hypertensive parents than in offspring of normotensive parents (75). ET-1 also contributes significantly to the resting vasomotor tone of diseased coronary arteries (76, 77).

Although ET_A blockade has been shown to prevent stress-induced myocardial infarctions in hypercholesterolemic mice (78), the relationship of ET-1 to MSI has not been described in humans. Nevertheless, the particular role of ET-1 in mediating vascular responses to stress suggests that it may play a role in causing MSI.

Endothelin-1, MSI, and the Sympathetic Nervous System

In patients with CAD, mental stress produces an increase in sympathetic tone that results in elevations of epinephrine and norepinephrine (7). These catecholamines have been directly implicated in contributing to cardiovascular pathology, as indicated by the ability of adrenergic blockade to reverse stress-induced endothelial damage in an animal model of chronic stress (79). Norepinephrine has an important role in local vasoconstriction during mental stress; it is released at local sympathetic termini in the coronary arteries, and it has a largely vasoconstrictive effect on vascular smooth muscle. Evidence increasingly points to a role for endothelial factors, and in particular ET-1, in mediating this vasoconstriction to norepinephrine.

ET-1 potentiates vasoconstriction to an infusion of norepinephrine in human arteries at concentrations of ET-1 that exert only a minimal direct pressor effect (80). This was observed in segments of resected human arteries *in vitro*, which exhibited contractile tension when exposed to norepinephrine that nearly doubled when a potentiating concentration of ET-1 was present. Significantly, these arterial segments were removed from patients undergoing CABG who likely did not have fully intact or functional coronary endothelium.

When the action of ET-1 is blocked in the human microcirculation *in vivo*, by pharmacologic blockade of the ET_A receptor using the specific receptor blocker BQ-123, the normal vasoconstriction observed during an infusion of exogenous norepinephrine is significantly attenuated (81). This was shown in a study using laser Doppler flowmetry to measure perfusion in the microcirculation of human skin. In the presence of BQ-123, the concentration of norepinephrine required to produce any decrease in perfusion was approximately three orders of magnitude greater than the minimal dose that would decrease perfusion when blockade of ET-1 receptors was absent. These findings were made in healthy subjects, and therefore the possibility exists that the sympathetic effect of ET-1 is much more pronounced in patients with diseased endothelium, in light of ET-1's significant contribution to vasomotor tone in diseased coronary artery segments (76). These findings also point to an interaction between ET-1 and norepinephrine, perhaps through a threshold effect in which critical levels of one molecule potentiate the effect of the other molecule. This interaction could then produce arterial vasospasm and vasoconstriction.

This possibility was explored in a study of variant or Prinzmetal angina in human subjects. Intracoronary infusions of acetylcholine and ergonovine, which has partial α-adrenergic agonist activity, were used to induce coronary vasospasm in patients with a history of variant angina. Subjects who suffered coronary vasospasm had higher circulating levels of ET-1 at baseline in both peripheral veins and the coronary sinus than those who did not (82). Interestingly, those subjects who suffered vasospasm also demonstrated a transient *decrease* in their ET-1 levels during the vasospastic episode that returned to baseline as the episode resolved. This was attributed to an undescribed feedback mechanism that attempts to limit ET-1 release and activity during pathologic events.

These results suggest a relevant role for ET-1 in mediating and potentiating the vasoconstrictive effects of sympathetic activity. They also suggest that sympathetic activation during mental stress may promote vasospasm and a reduction in coronary blood flow when underlying cardiovascular pathology, and in particular endothelial dysfunction, already exists. Sympathetic activation may do this both through adrenergic receptor stimulation and by stimulating the effects of ET-1 on the coronary circulation.

In summary, acute mental stress plays an increasingly recognized role in causing cardiovascular pathology. It can precipitate ACS and arrhythmia leading to sudden cardiac death. It can also cause transient, subclinical myocardial ischemia in vulnerable individuals, and the occurrence of this ischemia indicates a worse prognosis for the individual's cardiovascular disease. Those who suffer from MSI may have worse existing disease in their vessels than those who do not, or the phenomenon itself, brought about acutely but silently, may cause long-term damage that is undetectable in the acute

phase. There is evidence that MSI is related to both sympathetic neurohormonal and endothelial factors. Despite the clinical relevance of MSI, no noninvasive, easily administered test exists to document or predict its occurrence.

A method known as peripheral arterial tonometry (PAT) has been developed that measures the pulse wave amplitude (PWA) of the small peripheral arteries in the microcirculation of the distal fingertip. It is noninvasive and may be useful as a diagnostic test. Past studies have indicated that a value of 0.8 for the ratio calculated by this method may differentiate those with and without cardiovascular pathology (83, 84). This method has been shown to reflect sympathetic activation and vasoconstriction due to the rich innervation of α-adrenergic fibers in the vascular bed (85-88). However, recent results suggest that it correlates with endothelial function as well, as the change in peripheral PWA after brachial artery occlusion has been found to correlate strongly, in a linear fashion, with the traditional measurement of brachial arterial diameter during reperfusion of the occluded artery (89). These correlations of PWA measurements with both α-adrenergic tone and endothelial function suggest that they provide a complex, functional measure of the interrelated systems contributing to arterial tone. This study assesses the utility of the PAT method as an effective, noninvasive tool to diagnose or predict the occurrence of MSI.

STATEMENT OF PURPOSE, HYPOTHESIS, AND AIMS

The purpose of this study is to investigate the relationship between Mental Stress Ischemia (MSI) and changes in neurohormonal output during mental stress, as measured indirectly by PAT. It is possible that PAT could prove useful as an easily administered test to diagnose or screen for MSI. In order to better understand the pathophysiological mechanisms that may contribute to increased arterial tone during mental stress, the direct relationship between PAT and levels of ET-1 and catecholamines is also studied.

Hypothesis

Peripheral arterial tone, as measured by PAT, will have a significant relationship with MSI, indicated by a strong predictive relationship of PAT to MSI, with a normal PAT ratio (>0.8) occurring predominantly in those without MSI, and an abnormal PAT ratio (≤0.8) occurring in those with MSI. Levels of sympathetic catecholamines during mental stress and levels of ET-1 after mental stress will be significantly higher in those with an abnormal PAT ratio than in those with a normal PAT ratio.

Aims

1) To investigate the relationship between PAT ratio, a noninvasive index of peripheral arterial tone, and MSI, as measured by myocardial perfusion imaging.

2) To investigate the contributions of sympathetic catecholamines and ET-1 to increased peripheral arterial tone during mental stress, as measured by PAT.

METHODS

Subjects: Subjects for the study were recruited from patients receiving care at the Cardiology Clinics of Yale-New Haven Hospital, the Connecticut Veterans' Affairs West Haven Hospital, and affiliated clinics. Men and women from all ethnic backgrounds were recruited for the study. All subjects carried an existing diagnosis of stable CAD, based on either a positive exercise stress test or history of a coronary intervention (bypass surgery or therapeutic coronary catheterization).

Potential subjects were excluded from the study if they met any of the following criteria: MI or unstable angina in the preceding 6 months; CABG surgery or PTCA in the preceding 6 months; history of major cardiac arrhythmia, or use of a pacemaker or AICD; uncompensated heart failure; presence of any other incapacitating or life-threatening illness; major psychiatric disorder or abuse of any substance; use of benzodiazepines or other sedatives. Also excluded were pre-menopausal women with serum estradiol >50 pg/ml and women using hormone replacement therapy, due to the protective effects of estrogens on vascular function.

Enrolled subjects were classified as obese if their BMI was ≥30. Subjects classified as diabetic were those who carried a clinical diagnosis of Type I or II diabetes mellitus. Subjects with a recent history of systolic pressure >140 mm Hg or diastolic pressure >90 mm Hg were classified as hypertensive. Those with total cholesterol ≥200 mg/dl or LDL ≥130 mg/dl were classified as having hypercholesterolemia. Subjects' smoking history was also recorded.

Subjects were asked to eat a light breakfast and take their normal medications on the day of the study, and then report to the study center. Recruiting and subject enrollment were performed by the study coordinator.

Summary of Protocol: Subjects were brought to the neurocardiac research lab at the West Haven VA Hospital, and the study coordinator obtained informed consent. Demographics, including cardiovascular comorbidities and medications, were recorded for the subject using a questionnaire administered immediately after informed consent was obtained. IV access was secured, and then the subject underwent baseline myocardial perfusion imaging (MPI) using single-photon emission computed tomography (SPECT) with technetium-99-sestamibi injection. The subject was then placed in a relaxed, recumbent position, and a blood pressure cuff and ECG leads were placed. A PAT probe was also placed on the middle finger of the arm without BP cuff or IV catheter. Hemodynamics and pulse wave amplitude were recorded continuously throughout the experiment. The subject began the stress protocol by undergoing a relaxation period for 15 minutes, starting with a structured relaxation induction, to bring the subject to a relaxed baseline. During this time, blood was obtained for measurement of baseline catecholamines and ET-1.

After 15 minutes, the stress task began. The subject was instructed to recall a recent, emotionally charged situation that made them angry or irritated, and then to recount the specific details of the event to the interviewer. During this task, blood was drawn to quantitate the catecholamine surge associated with emotional activation, and another injection of technetium-99 isotope was administered to assess myocardial perfusion during MS. The stress condition persisted for approximately 10 minutes as the

subject continued to recount the stressful event, and then a final blood sample was drawn to measure stress levels of ET-1 after completion of the task.

Upon completion of the protocol, the IV catheter was withdrawn, all equipment and leads were removed, and the subject was thanked for his or her participation.

Stress Task: The experiment employed a relaxation period followed by an anger recall task. The relaxation period began with the interviewer asking the subject to describe a setting in which they felt completely relaxed. The interviewer then asked the subject to imagine himself in that location, and provided verbal cues to invoke the image of the place in the subject's mind. The subject then lay quietly with eyes closed for 15 minutes with no further conversation with any of the research staff, during which time baseline measurements were obtained.

The stress condition employed anger recall to induce a mentally stressful state in the subjects. This task is primarily an emotional stressor that has been reported as consistently producing hemodynamic activation as well as coronary arterial constriction in several studies (24, 25, 90). The anger recall task was chosen for the study over other laboratory mental stressors because it was found to be consistent in eliciting MSI in vulnerable subjects (91). Subjects were aroused from the relaxed state and asked to think of a recent even that had made them irritated, aggravated, or frankly angry. When they had thought of one, they were asked to take a few seconds to fix the details in their mind, and then recounted it to the interviewer. The interviewer would ask questions throughout the stress condition designed to get subjects to talk about things that they said, did, and felt during the stressful event, and during this time all stress data would be recorded and

the isotope administered. The condition and the study were ended as soon as all data and samples were collected, after approximately 10 minutes.

Relaxation was performed either by the research assistant or the research group's clinical psychologist. Either the author or the clinical psychologist performed the stress interview.

Hemodynamic Measurements: Subjects underwent continuous monitoring of heart rate, blood pressure, and ECG throughout the study. All measures were recorded at 5-minute intervals during the relaxation period and at 1-minute intervals during the stress task. Baseline hemodynamic parameters were calculated for each subject by taking the average of all measurements during the relaxation period. Parameters during mental stress were taken from the measurement that produced the greatest cardiovascular activation as measured by rate-pressure product (RPP), calculated as the product of HR and SBP at that time point. RPP is an accepted measure of cardiovascular activation, indicating reactivity to a mental or physical stressor and providing an index for cardiovascular demand created by a stress condition (92-94). The point at which a maximum for RPP was observed was used to determine the subject's HR and BP during MS. Hemodynamic measurements during the study were obtained either by the author or by the research assistant present. Calculation of values was performed by the author or by the research data assistant using methodology derived by the author.

SPECT-MPI: Baseline and stress scans of myocardial perfusion were obtained with sestamibi isotope injection as described, using standard methodology. All scans were performed in the Division of Cardiovascular Nuclear Imaging at the West Haven

VA Hospital. The technetium-99 isotope was generated and the myocardial perfusion scans were obtained by the Nuclear Medicine technical staff.

The two scans were compared to assess the occurrence of any reversible perfusion defect after undergoing the stress task. The presence of a defect was assessed qualitatively by one of the trained attending physicians in nuclear cardiology who was blinded to the subjects' other information; those with a perfusion defect were designated as positive for MSI, and those without a defect were designated as negative. A typical example of a positive scan is shown in Figure 1.

Administration of the isotope for the baseline scan was performed by the nuclear medicine staff. Either the author or the cardiology fellow participating in the study administered the isotope for the stress scan during the anger recall task.

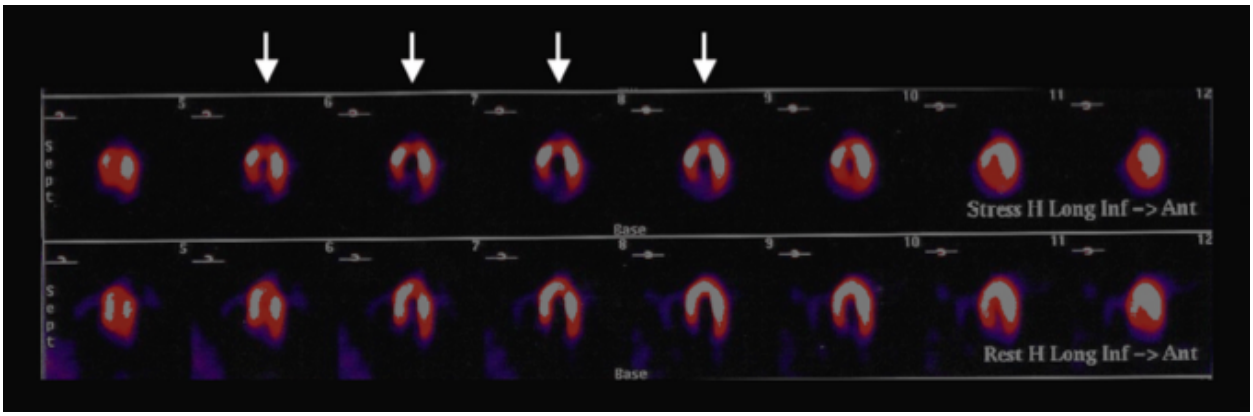

Figure 1. Typical example of SPECT-MPI scan positive for myocardial ischemia during mental stress. Stress images are in the upper row and baseline images are in the lower row. This subject displayed apical ischemia during mental stress, indicated by white arrows.

PAT Methodology: PWA was recorded using a peripheral arterial tonometer (PAT) (Itamar Medical, Israel). This instrument uses a noninvasive plethysmographic probe that places a uniform pressure field around the fingertip. The field is adjusted for the subject's baseline diastolic pressure to prevent venous pooling of blood and unload arterial wall tension, such that any volume changes in the fingertip with each pulse are only those of arterial perfusion (86). The probe is attached to a pressure transducer and through it to a main system. The main system amplifies the transducer signal and band-pass filters it in the frequency range of 0.3 to 30 Hz. The system then sends the signal to a laptop computer, which records the amplitude of each pulse wave as a continuous tracing and provides a measure of the small arterial smooth muscle tone in the fingertip. As tone increases, pulse wave amplitude decreases.

A ratio of the PWA during stress to the PWA before stress was calculated. This ratio was calculated by taking 1-minute representative segment from the relaxation period and a 30-second to 1-minute segment from the stress period with the lowest PWA, indicating maximum reaction to the stress condition. The presence of significant motion artifact necessitated the taking of specific segments from the stress condition, as the recounting of a stressful experience frequently caused subjects to tense, flex, or even move their hands and forearms. The PAT ratio provides an index of microarterial tone and function in the peripheral microcirculation. Figure 2 displays examples of PWA traces that had normal and abnormal PAT ratios.

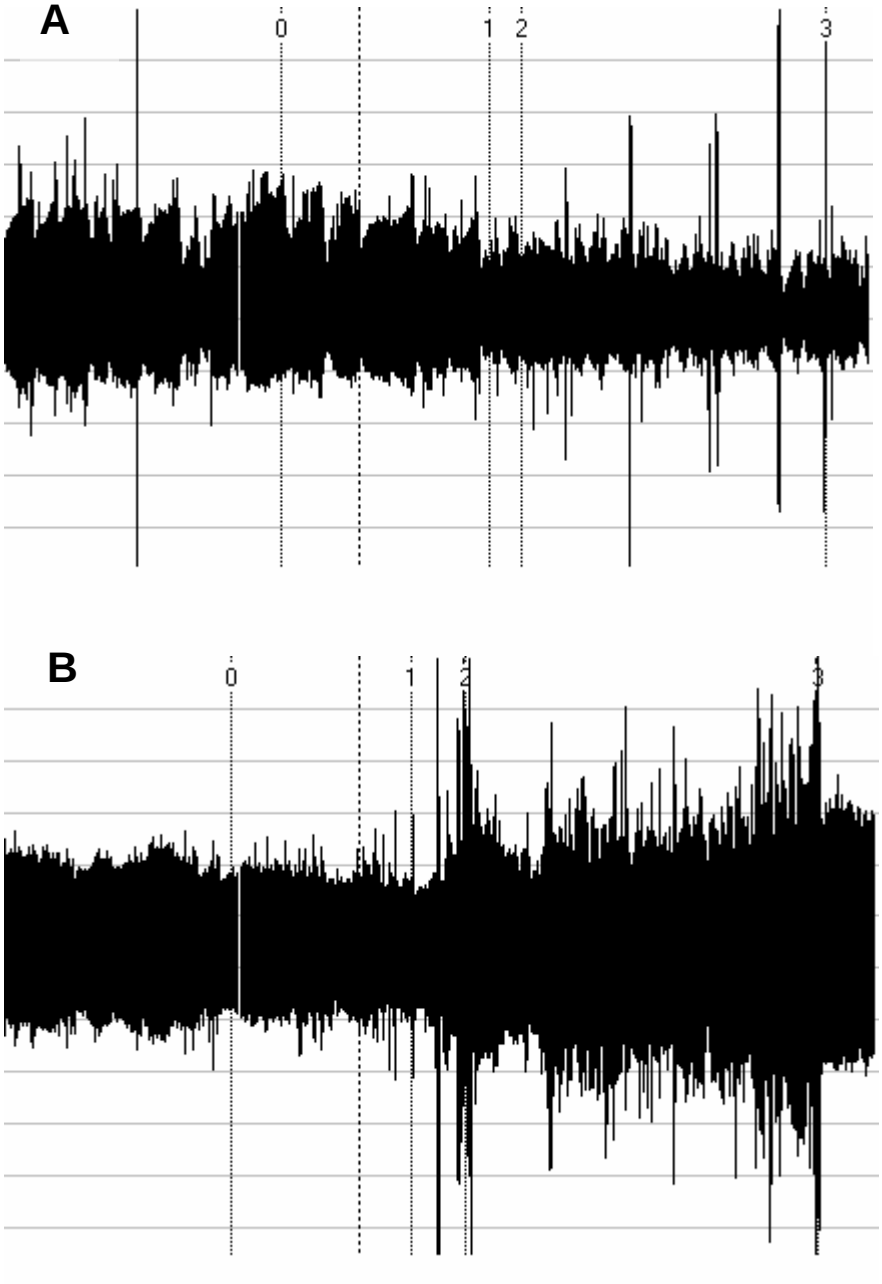

Figure 2. Examples of PWA traces with PWA displayed as a function of time. Numeral 0 in each trace indicates the relaxation period and numeral 1 indicates the beginning of the mental stress task. A. Trace resulting in an abnormal PAT ratio, with relative vasoconstriction during mental stress. B. Trace resulting in a normal PAT ratio, with relative vasodilatation during mental stress.

For the purposes of this paper, PAT will be used generically to describe the test and PAT ratio to describe the calculated result of the test, while PWA will indicate the physiological parameter that the test measures.

The recording of PWA during the study was performed either by the research assistant or by the author. All PAT ratios were calculated by the author.

Processing of Blood Samples: Blood was drawn from the IV line for analysis after 10 minutes of relaxation (baseline values), after 2 minutes of anger recall (stress catecholamine levels), and after completion of anger recall (stress ET-1 levels). All samples were collected into tubes using potassium EDTA as an anticoagulant except for those used for catecholamine analysis. Blood samples were kept on ice until centrifugation, which was performed promptly after completion of the study protocol. They were spun at 3000 rpm for 5 minutes and then the plasma was withdrawn and aliquotted. All aliquots were stored at -80°C until analysis. Either the author or the cardiology fellow drew all blood samples. The author processed all blood samples unless not available after the study, in which case they were processed by the research data assistant or the fellow.

Measurement of Catecholamines: Blood for catecholamine analysis was placed in special refrigerated tubes containing reduced glutathione to prevent oxidation and maintained at 0°C until processing immediately following the protocol. Samples were centrifuged as above and plasma aliquots were stored at -80°C until batch analysis. Epinephrine and norepinephrine were measured from plasma samples using reverse-phase high-performance liquid chromatography (ESA Inc, Chelmsford, MA) and electrochemical detection (Coulochem II) after alumina extraction. The intra-assay

coefficient of variation for this method is 1-2%, and the inter-assay coefficient of variation varied from 10% for norepinephrine to 25% for low levels of epinephrine (<25 pg/ml). All processing and analysis of catecholamine samples were performed by the staff of the Yale General Clinical Research Center.

Measurement of Endothelin-1: ET-1 was measured using enzyme-linked immunosorbent assay (ELISA). An ELISA kit using the sandwich amplification technique from Biomedica Gruppe (Vienna, Austria) was used that provides quantitative, colorimetric measurement of ET-1 levels in human plasma. Samples were run in duplicate for each subject and read on a Bio-Rad microplate optical density reader immediately upon completion of the assay. Plates were read measuring absorbance at 450 nm with reference measurement at 595 nm. Each plate included two different positive controls and two negative control wells in addition to the standard curve. Results from each batch were fit to a standard curve generated for each unique kit, and concentrations of each duplicate sample were averaged and reported in units of fmol/ml. In all cases, standard curves displayed an r-value for correlation >0.995, and positive controls were noted to be within the concentration range given for both controls. The manufacturer's median ET-1 concentration detected by the kit was reported as 0.34 fmol/ml (n=50). Cross-reactivity with other ET isoforms was reported as 100% for ET-2, <5% for ET-3 and <1% for big ET. Concentrations were converted into units of pg/ml using a conversion factor of 2.5, based on a molar weight of 2500 Da for ET-1. A correction factor of 0.8 was then applied to the concentration to correct for an estimated 20% of the reported concentration being attributable to ET-2 based on normal human ET-2 plasma levels, so that the final reported concentrations are 80% of the measured

concentrations. The author performed all analyses of ET-1 samples using the facilities and expertise of Dr. George Tellides.

Data Analysis: Subjects were placed in the ischemic or non-ischemic groups based on the results of their SPECT-MPI studies. Age was compared for each group using the Student's *t* test, while LVEF, which was bimodally distributed in the ischemic group, was compared using the Wilcoxon rank-sum test. Demographics for each group were compared using Fisher's Exact Test for cross-tabulation. All BP data were normally distributed and comparisons were made using the Student's *t* test. HR, RPP, catecholamine levels, and ET-1 levels were not normally distributed and exhibited significant rightward skewing in the most reactive individuals from each group. As a result, nonparametric tests were used for comparisons: the Wilcoxon signed-rank test for intragroup comparisons and the Wilcoxon rank-sum test for intergroup comparisons. Additional comparisons were performed to compare groups with positive and negative PAT test results. P-values <0.05 were considered significant. Average PAT ratios are reported as mean ± SEM. All other results are reported as mean ± SD except where data are not normally distributed, in which case they are reported as median (interquartile range).

The average PAT ratio was compared between MSI-positive and MSI-negative groups using the Student's *t* test. A receiver operating characteristics (ROC) curve was generated for the relationship between PAT ratio and MSI to find a threshold test value for PAT ratio with maxima of sensitivity and specificity. ROC results were compared with existing findings that suggest a threshold of <0.8 for an abnormal PAT ratio (83). A group of those at or below the threshold for an abnormal ratio was determined using the

results of the ROC curve and analysis. Cross-tabulation of PAT test results with SPECT-MPI results was performed to evaluate significance and concordance of the test; these were assessed using Fisher's Exact Test.

The reported demographics included several comorbidities that can adversely affect vascular function, as well as medications that are protective of vascular function. Accordingly, additional ROC curves were generated to compare the performance of the PAT ratio based on the presence or absence of these comorbidities and medications. Hypercholesterolemia, hypertension, and the use of aspirin, β-blockers, and statins were all too prevalent in the study group to be able to have adequate comparison groups. Obesity, active smoking, diabetes, and ACE inhibitor use all had lower prevalence, and comparisons were generated for these demographic variables.

Spearman correlation routines were performed for all neurohormonal levels to assess the contribution of each mediator to PAT ratio. Those that appeared to have strong correlations were assessed for a predictive relationship with PAT ratio using univariate least-squares linear regression. Results are reported with the Spearman coefficient and P-value for the slope of the regression model.

The author performed all statistical analyses with the technical and intellectual assistance of Drs. Soufer and Burg and Dede Collins, the research group's biostatistician. All analyses were done with either NCSS97 or SAS v.8.2 statistical software.

Statement of Participation: The simultaneous measurement of so many different sources of data required that several individuals participate in each successful study and that studies take place regularly during the week for over a year. However, the author was the primary investigator responsible for acquisition and analysis of the data in this

study. The author also participated extensively in the design and performance of the methods, specifically those for PAT measurements, analysis of ET-1 levels, and attendant blood samples.

RESULTS

In total, 92 subjects were recruited for the study. Fifteen of these were excluded from the study either because they were unable to complete the protocol or due to technical problems with either their PAT readings or their perfusion imaging scans. The study group included 77 subjects who completed the protocol and had complete hemodynamic, PAT, and SPECT results, 26 of whom demonstrated mental stress ischemia on SPECT-MPI and 51 of whom did not. The prevalence of MSI in the study population was 34%, which is consistent with other studies that have used anger recall to provoke MSI. Of this group, 68 had complete catecholamine data. Nine subjects failed to have data due either to insufficient quantities of blood samples or hemolyzed samples, or to technical difficulties running the samples. Of the final study group, 39 had complete ET-1 data. Reasons for not having data included those for incomplete catecholamine data. In particular, the first 15 subjects did not have sufficient blood collected to run ET-1 or inflammatory markers, as the assays were added to the protocol after this time. Additionally, 12 subjects had to be excluded due to cross reactivity of an unknown component of their plasma with the ELISA kit primary antibody, leading to extremely and inappropriately elevated optical absorptions of their samples. They were all found to display this reactivity even on repeat runs on different plates, and in the setting of normal standard curves and controls. In summary, the final study group comprised 77 patients with PAT and SPECT-MPI data which were used for all subsequent analyses, and the prevalence of MSI in this group was 34%.

Table 1. Demographics of the study group.[A]			
Variable	Overall (n=77)	Ischemic (n=26)	Non-Ischemic (n=51)
Age (years)	65.6 ± 8.6	64.5 ± 6.9	66.4 ± 9.4
Female	8 (10)	1 (4)	7 (14)
Nonwhite	13 (17)	5 (19)	8 (16)
LVEF (%)	54 (17)	51 (18)	55 (14)
Comorbidities			
Hypertension	67 (87)	23 (88)	44 (86)
Diabetes	21 (27)	9 (35)	12 (24)
Hypercholesterolemia	74 (96)	26 (100)	48 (94)
Obesity	28 (36)	10 (38)	18 (35)
History of Smoking	53 (69)	20 (77)	33 (65)
Actively Smoking	17 (22)	4 (15)	13 (25)
Medications			
ACE Inhibitor	40 (52)	14 (54)	26 (51)
Beta Blocker	60 (78)	20 (77)	40 (78)
Statin	69 (90)	23 (88)	46 (90)
Aspirin	54 (70)	18 (69)	36 (71)

[A] Values are displayed as n (%), mean ± SD (age), or median (interquartile range) (LVEF). There were no significant differences between the ischemic and non-ischemic groups with regard to demographics and comorbidities (P = NS).

Demographics from the study group are shown in Table 1. The great majority of subjects were white males despite a sustained effort to recruit women and minorities. The two groups (with and without MSI) did not differ significantly with respect to age, LVEF, or any cardiovascular comorbidity. The groups also did not differ with respect to use of any cardiovascular medications; all medications were present in nearly identical proportions.

Baseline hemodynamics and responses to mental stress, including heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and RPP, are shown in Table 2. There were highly significant increases in all hemodynamic parameters during mental stress for both ischemic and non-ischemic groups ($P < 0.001$). However, there was no significant difference between the two groups with respect to any of the parameters measured.

Table 2. Hemodynamics at baseline and hemodynamic responses to mental stress.[A]

Variable	Overall (n=77)	Ischemic (n=26)	Non-Ischemic (n=51)
Baseline			
HR (bpm)	56 (11)	56 (11)	56 (13)
SBP (mm Hg)	134 ± 17	134 ± 21	134 ± 16
DBP (mm Hg)	75 ± 10	73 ± 9	75 ± 10
MAP (mm Hg)	94 ± 11	94 ± 11	95 ± 10
RPP (bpm*mm Hg)	7164 (2580)	6797 (3411)	7198 (2316)
Mental Stress			
HR (bpm)	66 (14)	64 (19)	68 (12)
SBP (mm Hg)	162 ± 22	158 ± 24	164 ± 20
DBP (mm Hg)	89 ± 11	87 ± 12	91 ± 10
MAP (mm Hg)	114 ± 12	110 ± 14	115 ± 11
RPP (bpm*mm Hg)	10800 (2705)	9712 (5733)	11200 (2496)

[A] Values are displayed as mean ± SD or median (interquartile range). All groups experienced highly significant increases in all hemodynamic parameters during mental stress compared to baseline conditions ($P < 0.001$). There were no significant differences between the ischemic and non-ischemic groups at similar conditions or from baseline to mental stress (P = NS).

The average PAT ratio for the overall study group was 0.86 ± 0.04, indicating that, on average, vasoconstriction occurred during the stress condition. A comparison of the average PAT ratio between the groups with and without MSI showed that those who experienced ischemia had an average ratio of 0.76 ± 0.04, while those who were without ischemia had an average ratio of 0.91 ± 0.05 (P = 0.03, Figure 3).

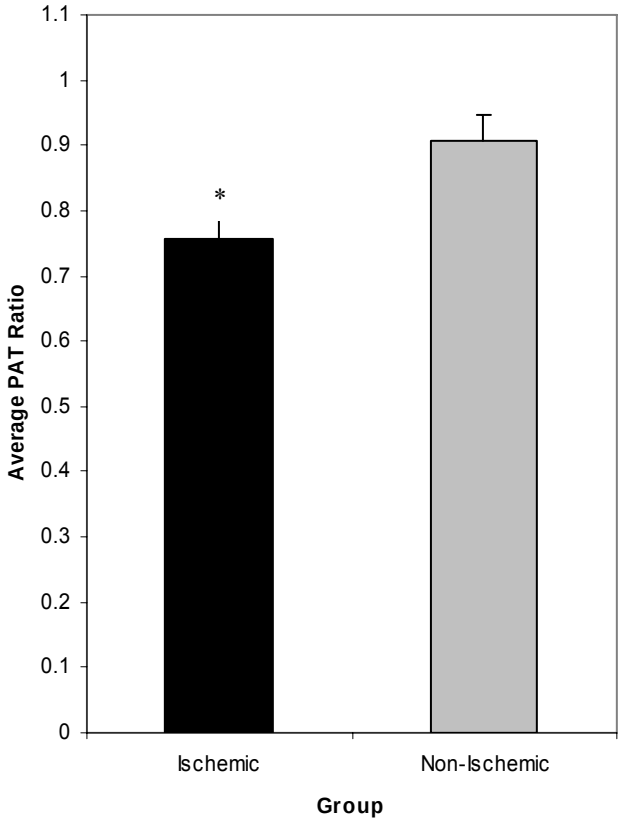

Figure 3. Average PAT ratio for those with and without mental stress ischemia. Those with ischemia had an average ratio of 0.76 ± 0.04 while those with no ischemia had an average ratio of 0.91 ± 0.05 (* P = 0.03 vs. non-ischemic group; bars indicate SEM).

To determine the optimum threshold of this measurement for diagnosing MSI, an ROC curve was generated for PAT ratio predicting MSI (Figure 4). The estimated area

under the curve (AUC) was 0.613 with a standard error of 0.065 (one-sided P = 0.04). This curve produced a maximum of sensitivity versus 1-specificity at a value of 0.78 to define a positive test, with a sensitivity of 0.62 and a specificity of 0.63 at this value. This threshold produced a positive predictive value of 0.46 and a negative predictive value of 0.76.

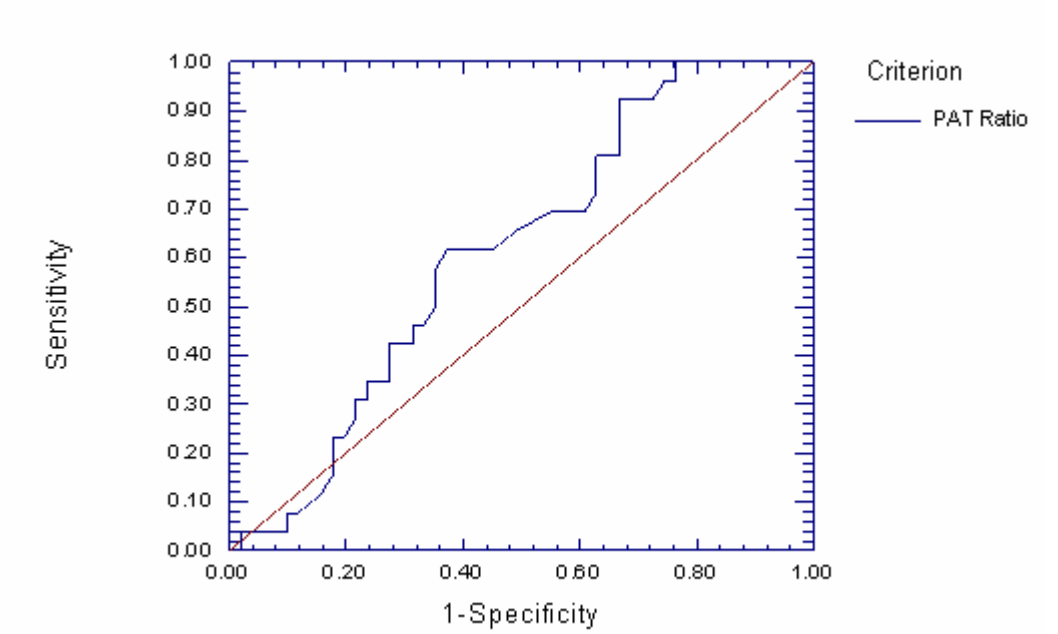

Figure 4. ROC curve for PAT ratio predicting mental stress ischemia. The AUC is 0.613 (SE, 0.065, one-sided P = 0.04), indicating that PAT ratio has diagnostic utility in predicting MSI.

In order to account for the effects of ACE inhibitors on hemodynamics, we analyzed these results based on the presence or absence of ACE inhibitors in the study group. When an ACE inhibitor was present, the AUC was 0.768 (standard error 0.081, one-sided P < 0.001) versus 0.408 (standard error 0.095, one-sided P = 0.17) when an ACE inhibitor was absent (P = 0.004 for difference in AUC, Figure 5). In the group

taking an ACE inhibitor, the sensitivity and specificity of the test jumped to 0.86 and 0.73, respectively, at the established threshold of 0.78. These values were also maxima for the ROC curve. This discrepancy in the performance of PAT ratio as a test was seen despite the fact that the average PAT ratio among those taking an ACE inhibitor was 0.88 ± 0.07 and was not significantly different than the average ratio among those not taking an ACE inhibitor, which was 0.83 ± 0.04 (P = 0.48). There were no significant differences in AUC when comparing active smoking, obesity, and diabetes (data not shown).

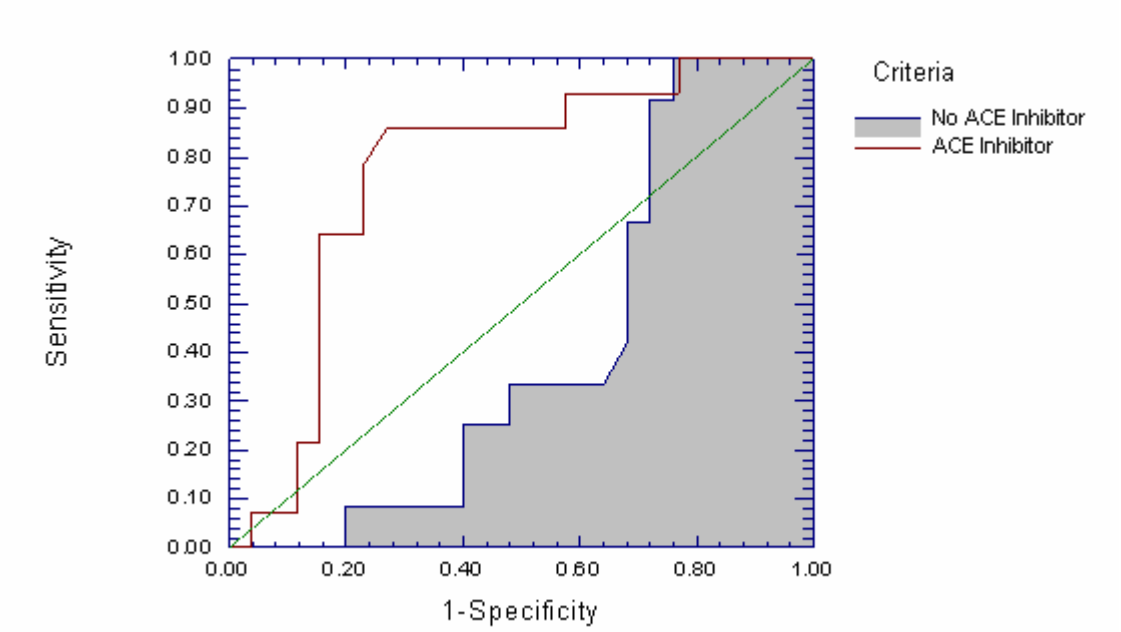

Figure 5. ROC curves for PAT ratio predicting mental stress ischemia for those who were taking ACE inhibitors versus those who were not. The AUCs are 0.768 (SE = 0.081, one-sided P < 0.001) when ACE inhibitors are present and 0.408 (SE = 0.095, one-sided P = 0.17) when they are absent (P = 0.004 for difference in AUC). This indicates that PAT ratio performs significantly better as a test in those who are taking ACE inhibitors.

The threshold of 0.78 resulting from the ROC analysis was used to divide the entire study group into groups of those either above or below the PAT ratio threshold. Cross-tabulation of these groups with the ischemic and non-ischemic groups showed that 47% of those with a PAT ratio equal to or lower than the threshold had ischemia during mental stress, while only 23% of those with a ratio greater than the threshold had ischemia. The overall concordance of the test was 64%, i.e., the test properly diagnosed 64% of the subjects (P = 0.03, Table 3).

Table 3. Cross-tabulation of mental stress ischemia with results of PAT testing for the entire study group.[A]

	Ratio ≤ Threshold	Ratio > Threshold	(Totals)
Ischemic	16	10	26
Non-Ischemic	18	33	51
(Totals)	34	43	77

[A] The relationship between PAT and mental stress ischemia was significant (P = 0.03).

As indicated by the ROC results, when only subjects on an ACE inhibitor were tabulated, these numbers improved significantly. Sixty-three percent of those at or below the threshold had ischemia, while only 10% of those above the threshold had ischemia, yielding a concordance of 78% (P < 0.001, Table 4). Among those subjects not taking an ACE inhibitor, concordance of the two measures was only 49% (P = 0.72, data not shown).

Table 4. Cross-tabulation of mental stress ischemia with results of PAT testing for subjects taking an ACE inhibitor.[A]

	Ratio ≤ Threshold	Ratio > Threshold	(Totals)
Ischemic	12	2	14
Non-Ischemic	7	19	26
(Totals)	19	21	40

[A] The relationship between PAT and mental stress ischemia for those taking an ACE inhibitor was highly significant ($P < 0.001$).

In order to investigate relationships between catecholamines and PAT ratio, the correlations between them were analyzed. Among the entire study group, a negative correlation was found between changes in norepinephrine level during mental stress and PAT ratio. Linear regression of the two variables showed a predictive relationship of change in norepinephrine for PAT ratio ($r = -0.367$, $P = 0.002$). As the magnitude of change in norepinephrine increased, the observed PAT ratio tended to decrease. This is consistent with the previously documented relationship between α-adrenergic stimulation and reduction in PWA. In contrast to this, there was no significant correlation observed between epinephrine levels and PAT ratio, and change in epinephrine did not correlate significantly with PAT ratio ($r = 0.179$, $P = 0.14$).

Catecholamine levels for groups above and below the PAT threshold were compared (Figure 6). The levels of norepinephrine in the group with PAT ratios at or below the threshold (≤0.78) were lower at baseline (235 (221) pg/ml vs. 290 (210) pg/ml) and during mental stress (259 (194) pg/ml vs. 294 (311) pg/ml) than the above-threshold (>0.78) group, although not significantly so. However, the group at or below the threshold displayed a significant increase in norepinephrine from baseline to mental

stress (P = 0.007) while levels in the above-threshold group did not change significantly. Increases in epinephrine from baseline to mental stress were significant for both groups (P = 0.02 for those at or below threshold, P = 0.002 for those above threshold, data not shown).

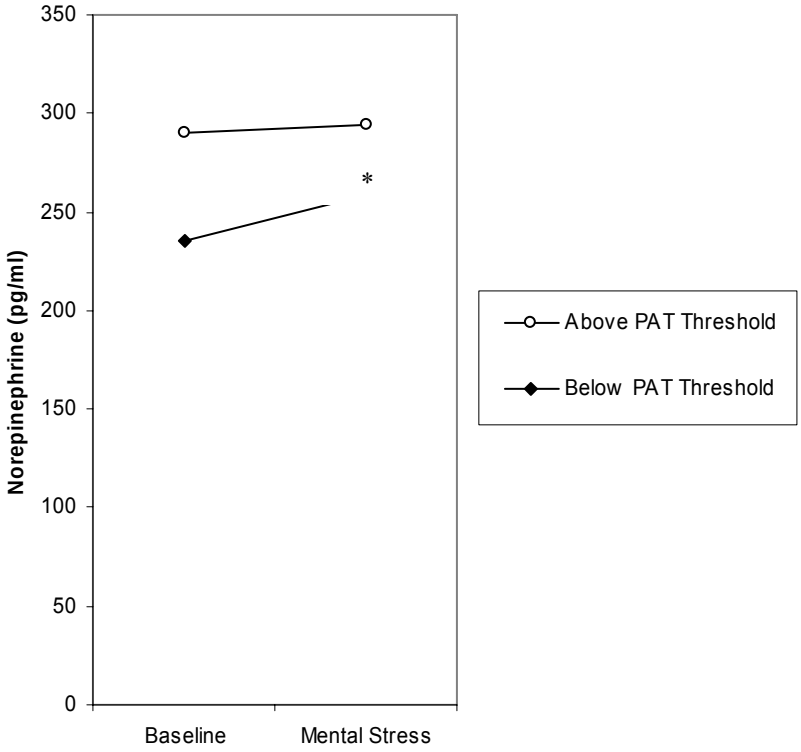

Figure 6. Median norepinephrine levels (pg/ml) at baseline and during mental stress. Subjects with an abnormal PAT ratio at or below threshold (≤0.78) displayed a significant increase in norepinephrine levels from baseline to mental stress, while those with a ratio above threshold did not (* P = 0.007 vs. baseline for those below threshold).

Endothelin-1 levels were compared between PAT groups as well (Figure 7). ET-1 levels did not differ significantly between those with ratios at or below threshold (≤0.78) and those with ratios above threshold (>0.78) at any time point. The group at or below threshold had levels of 0.90 (1.20) pg/ml at baseline and 0.97 (1.59) pg/ml immediately after mental stress, and these increased to 1.04 (1.51) pg/ml by 24 hours after mental stress. These levels did not differ significantly from one another. In contrast, the group above threshold had levels of 0.97 (1.29) pg/ml at baseline and 1.15 (1.34) pg/ml after mental stress, and then experienced a significant drop to 0.93 (1.61) pg/ml after 24 hours ($P = 0.01$). The overall change in ET-1 from after mental stress to 24 hours later displayed a negative correlation with PAT ratio that was not significant but approached significance ($r = -0.294$, $P = 0.07$).

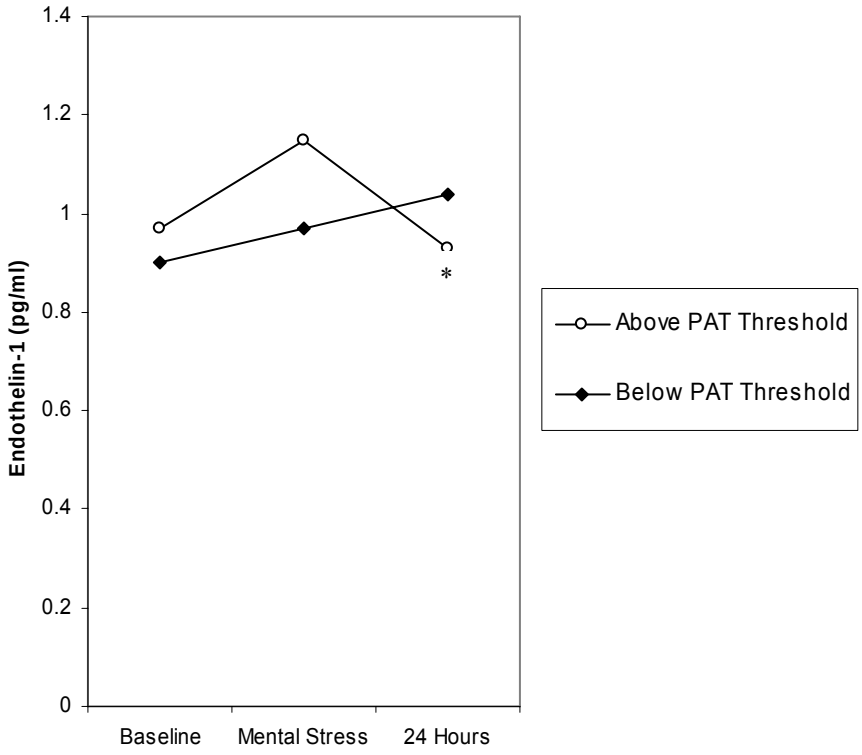

Figure 7. Median ET-1 levels (pg/ml) at baseline, immediately after mental stress, and 24 hours after mental stress. Those with a normal PAT ratio above the threshold (>0.78) displayed a significant decline in ET-1 levels after 24 hours (* P = 0.01 vs. mental stress for those above threshold). Conversely, those with an abnormal PAT ratio below threshold (≤0.78) displayed a slight increase in levels at 24 hours that was not significant.

DISCUSSION

In this study, we have demonstrated that an abnormal PAT ratio during mental stress is strongly correlated with the presence of MSI as documented by SPECT perfusion imaging. An abnormal PAT ratio ≤0.78 correctly identified 62% of those subjects vulnerable to MSI, while a normal ratio >0.78 correctly identified 65% of those not vulnerable to MSI. Subjects who had an abnormal PAT ratio also demonstrated a neurohormonal profile that distinguished them from those with a normal ratio, marked by a significant increase in norepinephrine levels during mental stress and maintenance of ET-1 levels 24 hours after mental stress. Acute mental stress causes changes in neurohormonal output, as measured indirectly by PAT. These changes lead to increased arterial tone that is more pronounced in those who are vulnerable to MSI, suggesting a mechanism by which myocardial ischemia may occur during mental stress.

Mental stress ischemia indicates a poor prognosis in those who are vulnerable to it. It likely occurs in such individuals on a daily basis and leads to cardiovascular disease progression and increased mortality. Yet it is becoming clear that it is amenable to specific therapies, and that, for those in whom it is detected and treated, such therapy can have significant benefits (8-11). MSI is not easy to diagnose with most current methodologies, and considerations of cost and risk to the patient make those that are definitive unsatisfactory for routine use. The availability of an easily administered test for MSI would enable screening of at-risk patients as a component of good cardiovascular care. Those who are identified as susceptible to MSI would then be candidates for specific interventions that could positively impact their survival.

In screening patients for MSI using the PAT method, those with known CAD would undergo the test to assess vulnerability to MSI. Patients who displayed an abnormal ratio would then be considered for a study using perfusion imaging to document definitively the occurrence of MSI, contingent upon other clinical issues and risk factors particular to each patient. For those patients found to be vulnerable to MSI, management of mental stress and emotional factors would become an integral part of the management of their CAD.

In order to assess the performance of PAT as such a screening test, ROC data were generated to find an optimum threshold at which to distinguish a normal PAT ratio from an abnormal one, in other words, a threshold that would produce the strongest relationship between PAT and MSI. In a previous paper, Goor and colleagues looked at PAT ratio and mental stress testing in 16 subjects with known coronary disease, using presence of either ventricular wall motion abnormality or drop in LVEF as an indicator of MSI (84). The study used a threshold of 0.80 or less to define an abnormal PAT ratio, derived from a previously published ROC study (83). At this threshold, they reported a concordance of 88% between categorical PAT results and the presence or absence of MSI. The threshold of 0.78 derived for this study is very similar to the published threshold of 0.80 even though that threshold was initially derived in a model of exercise stress perfusion imaging. This suggests that both exercise stress and mental stress may exploit similar mechanisms in causing ischemia. This study differs from previous studies in that 77 subjects were assessed using a "gold standard" method for observing myocardial ischemia—SPECT-MPI. Furthermore, the current study considers multiple comorbidities and medical therapies in this assessment. However, the overall

concordance of 64% seen in the present study, while significant, was well below the 88% reported previously.

A possible explanation for this discrepancy is the use of LV function as the index for MSI in the study by Goor and colleagues, versus the use of SPECT perfusion imaging in this study. By using LV function, the previous study would likely have observed those who experienced a decrement in function due to segmental ischemia as well as those who experienced such a decrement because of vasoconstriction and afterloading. Both of these phenomena would likely then have correlated with PAT ratio, strengthening the overall concordance.

This discrepancy in concordance also points to a similar observation regarding the relationship between subjects who were taking ACE inhibitors and those who were not. It is notable that ACE inhibitor therapy increased both the sensitivity and specificity of the PAT ratio. This therapy may decrease the false-negative rate by selecting for those subjects vulnerable to MSI who have an exaggerated sympathetic response to mental stress. This may perhaps be the case because of an undescribed association of this response with hypertension. This exaggerated response then causes peripheral vasoconstriction and MSI in vulnerable individuals despite treatment with ACE inhibitors, as opposed to those who may experience MSI without peripheral vasoconstriction. The false-positive rate may be improved by a decrease in nonspecific peripheral vasoconstriction caused by angiotensin II in those taking ACE inhibitors. Clearly, a great deal of work remains to be done in order to understand fully the various factors that contribute to the relationship between MSI and PAT, so it can generate the maximum clinical benefit. Both peripheral arterial tone and myocardial perfusion depend

on complex, multifactorial mechanisms that involve the interplay of both physical forces and a multitude of biological molecules. These include many that were not investigated at all by the present study but likely play an important role in MSI and PAT, such as NO, prostaglandins, and inflammatory cytokines.

Nevertheless, the current study provides some findings that may contribute to the understanding of these mechanisms. The overall correlation of PAT ratio with changes in norepinephrine levels and the significant increase in norepinephrine seen preferentially in those with an abnormal ratio are unsurprising given previous work that has linked PAT ratio to sympathetic output. However, those with a normal ratio experience a significant decline in their circulating ET-1 levels after 24 hours, while those with an abnormal ratio do not. While circulating ET-1 may rise transiently after a mental stressor, prolonged endothelial dysfunction that is sensitive to endothelin receptor blockade may also result from mental stress (72). Most ET-1 is contained in the vessel wall, in the abluminal space between the endothelium and VSMCs, where it is bound to receptors. Both the transient rise in levels during mental stress, as well as any sustained release of ET-1, likely result from spillover of ET-1 from this space into the circulation, which may cause the failure of circulating levels to decline after mental stress in some individuals. This may help to explain why Kuvin and colleagues reported that, in a group of 89 subjects being evaluated for angina, PAT ratio during reactive hyperemia correlated strongly with a traditional measure of endothelial dysfunction, FMD of the brachial artery (89). It provides further evidence that PAT ratio provides an index not only of sympathetic tone but also of endothelial dysfunction. It is even possible that those who display a sustained elevation of ET-1 levels may ruminate more on the events discussed in the anger recall

task, leading to continued endothelial dysfunction and ET-1 release 24 hours later. These subjects may in turn be more susceptible to stress-induced vasoconstriction, which is then detected by PAT.

This study also makes some important observations about the phenomenon of MSI itself. The demographics and medication profiles of the ischemic and non-ischemic groups are essentially indistinguishable. This indicates that even in a population of patients with chronic stable CAD, a significant portion of whom have their risk factors medically controlled, a third or more of them will remain vulnerable to MSI. The presence of medications that protect against traditional risk factors does not guarantee protection against MSI, which underscores the need both for mental stress testing and for specific interventions to target MSI.

One of the potential causes of MSI may be an increase in myocardial oxygen demand due to the stress response. When this demand goes unmet in vulnerable subjects, they may become ischemic, display abnormalities of ventricular wall motion, or suffer a drop in LVEF. However, this study suggests that MSI, at least as observed with SPECT-MPI, is not principally related to myocardial demand. This is indicated by the lack of a significant difference in RPP, a classic index of myocardial oxygen demand, between the ischemic and non-ischemic groups; median RPP was in fact lower during stress in those vulnerable to MSI, although not significantly so. This finding suggests that other factors in addition to myocardial demand are responsible for producing MSI.

Such factors may vary significantly from person to person based on gender, race, and genetics. These findings were generated in a group largely composed of white males, and the relationship between MSI and PAT may be quite different in women and in

people of non-white racial backgrounds. Additionally, comorbidities that were too prevalent in this study for meaningful comparisons, such as hypertension and hyperlipidemia, need to be considered. Finally, the effect of ACE inhibitor therapy on the performance of the test indicates that its performance needs to be studied in groups in which the presence or absence of other vasoactive medications, such as β-blockers, can be assessed.

Ultimately, the biological and demographic factors that affect PAT and MSI need to be integrated into models that can describe how changes in PWA during mental stress occur under various conditions and in various patient groups. This will allow PAT to provide the maximum utility for mental stress testing and is a necessary step for adoption of the technology to clinical applications. The possibility that such testing might become widely available offers the promise of effectively diagnosing and treating those who are susceptible to MSI, with important implications for the management and outcomes of patients with CAD.

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